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Effects of Triethylene Glycol on 'Mysidopsis bahia' (Crustacea: Mysidacea) and 'Menidia peninsulae' (Pisces: Atherinidae)

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16. ABSTRACT

Chronic effects of triethylene glycol (TEG), a chemical frequently used as a carrier-solvent in toxicity tests, were investigated in a 23-day life-cycle toxicity test with a mysid (Mysidopsis bahia) and in a 28-day early life-stage toxicity test with the tidewater silverside (Menidia peninsulae). We concluded that no direct adverse effect should be expected as a result of using TEG at concentrations as high as 100,000 times (mysids) and 360 times (tidewater silversides) those recommended in ASTM drafts of standard practices (< 0.01 µL/L in Mysidopsis bahia life-cycle tests and < 0.01 µL/L in tests with early life-stages of Menidia peninsulae) and that either a seawater or a TEG control may not be necessary.

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Effects of Triethylene Glycol on *Mysidopsis bahia* (Crustacea: Mysidacea) and *Menidia peninsulae* (Pisces: Atherinidae)

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ABSTRACT: Chronic effects of triethylene glycol (TEG), a chemical frequently used as a carrier-solvent in toxicity tests, were investigated in a 23-day life-cycle toxicity test with a mysid (*Mysidopsis bahia*) and in a 28-day early life-stage toxicity test with the tidewater silverside (*Menidia peninsulae*). Test organisms were exposed to the same range of concentrations: seawater control, 3.2, 10, 32, 100, 320, and 1000 mg/L. Effects, if any, were measured by survival and reproduction for the mysids and survival and growth for the silversides. We concluded that no direct adverse effect should be expected as a result of using TEG at concentrations as high as 100 000 times (mysids) and 360 times (tidewater silversides) those recommended in ASTM drafts of standard practices (≤ 0.01 μL/L in *Mysidopsis bahia* life-cycle tests and ≤ 0.01 μL/L in tests with early life-stages of *Menidia peninsulae*) and that either a seawater or a TEG control may not be necessary.

KEY WORDS: life cycles, aquatic biology, toxicity, chronic toxicity, triethylene glycol, *Mysidopsis bahia*, mysid, *Menidia peninsulae*, tidewater silverside, aquatic toxicology

Carrier solvents used in aquatic toxicity tests should not cause adverse effects on test animals. A number of solvents are being used in acute and chronic tests for which standard methods have been established for several freshwater and marine species [*1,2*]. Standard methods, which allow use of

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low concentrations of carriers, do not define maximum concentrations that can be used without direct toxicity of the solvent. Triethylene glycol (TEG) is one such carrier solvent. TEG is widely used in freshwater and marine toxicity tests as a solvent for polar and nonpolar compounds. TEG, being a slightly polar compound, is able to dissolve polar and nonpolar compounds and remain soluble in water. Acute and chronic effects of TEG on freshwater species, fathead minnows (*Pimephales promelas*), brook trout (*Salvelinus fontinalis*), and bluegill (*Lepomis machrochirus*), were determined by Cardwell et al [*3*] with flow-through systems. Taub and Harrass [*4*] evaluated the effect of single and repeated additions of TEG to static, 3.8-L (1-gal) microcosms containing freshwater species including algae, rotifers, and crustaceans. To provide similar data for estuarine species, we examined possible chronic toxicity of TEG in a life-cycle toxicity test with the estuarine mysid, *Mysidopsis bahia*, which is commonly found in protected or enclosed bodies of water, and the toxicity of TEG to the tidewater silverside, *Menidia peninsulae*, in an early life-stage toxicity test. These species were selected because they are frequently used in toxicity tests [*5,6*] and because they are easy to culture through all ontogenetic stages in the laboratory.

Materials and Methods

Toxicity tests were performed in accordance with ASTM proposed standard practices for mysids [*7*] and tidewater silversides [*8*]. Mysids were exposed for 23 days to a range of concentrations during which survival, number of reproductively active females, day of release of the first brood, and number of young in the first brood per female were monitored. Tidewater silversides were exposed to the same range of concentrations as the mysids, during which survival of embryos to hatching, survival of fry, and fish weight at 28 days were monitored.

Temperature was maintained at 25 ± 2°C and salinity at 20 ± 2 parts per hundred during culturing and testing at the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Gulf Breeze, FL. Seawater, pumped from Santa Rosa Sound, was filtered (20-μm filter), aerated, heated in a reservoir to the required temperature, and delivered to test aquaria located in a 25°C recirculating water bath. Salinity was maintained by dilution of natural seawater with deionized freshwater. The light regime was 14L:10D.

Mysid exposure aquaria were divided into three isolated chambers (13-L capacity). Siphons delivered 15 L/h to splitter boxes, which delivered 5 L/h to each chamber. TEG was added to seawater before its delivery to splitter boxes with Harvard Syringe Pumps[®3] for the lower concentrations and Milton Roy

[3]Harvard Syringe Pumps is the trademark of Harvard Apparatus Co., Inc., 150 Dover Rd., Millism, MA 02054, Model 935. Reference to commercial products does not constitute endorsement by the University of West Florida.

Pumps®[4] for the two higher concentrations. Controls received seawater without TEG. Water level in each chamber was fluctuated between 11.5 and 2.5 cm in depth approximately twelve times per day by self starting siphons. Dissolved oxygen and pH were monitored every fourth day for both tests with levels remaining within acceptable ranges (Table 1).

Twenty-four-hour old mysids used to start the test were laboratory-reared. Test exposure baskets, consisting of 15 by 2-cm petri dishes fitted with 13-cm-high cylinders of 363-μm nylon screen, were placed randomly in three rows of seven each. A computer program was designed to randomize placement of cups. Mysids were loaded, four per basket, until each basket in a row contained four mysids. This was repeated for each row until each basket contained 16 mysids, During tests, mortality of mysids and clogging of the baskets were monitored daily. Baskets were replaced weekly to prevent clogging of the screen. Mysids were transferred with a wide bore pipet to the clean cup. Mysids were fed twice daily with 1 mL of concentrated *Artemia* nauplii (24 to 32-h post-hydration of cysts). At sexual maturity, as indicated when females became ovigerous or when males developed claspers, pairs were placed in brood cups constructed from 10- by 1.5-cm petri dishes fitted with 13-cm-high cylinders of 363-μm nylon mesh. Cups were observed daily to determine time of brood release and number of young released in the first brood. As with the baskets, clean cups were introduced weekly.

To begin the early life-stage toxicity test, tidewater silverside embryos were obtained using the methods of Middaugh et al [*9*]. Embryos at Stage 10 [*10*] were collected from filter floss on which spawning occurred, fibrils were removed, and they were randomly placed in incubation cups identical to the

TABLE 1—*Average dissolved oxygen and pH values observed during chronic exposure of* Mysidopsis bahia *(23 days) and* Menidia peninsulae *(28 days) to triethylene glycol.*

Nominal TEG Concentration, mg/L	*Mysidopsis bahia*		*Menidia peninsulae*	
	Oxygen, mg/L	pH	Oxygen, mg/L	pH
Seawater control	7.2 (0.2)[a]	7.83 (0.13)[a]	7.1 (0.1)[a]	7.69 (0.10)[a]
3.2	7.2 (0.2)	7.77 (0.25)	7.1 (0.1)	7.73 (0.09)
10	7.1 (0.3)	7.83 (0.24)	7.1 (0.1)	7.71 (0.12)
32	6.9 (0.5)	7.81 (0.27)	7.2 (0.1)	7.73 (0.11)
100	7.0 (0.4)	7.81 (0.29)	7.1 (0.2)	7.73 (0.10)
320	6.8 (0.4)	7.81 (0.28)	7.2 (0.2)	7.73 (0.09)
1000	6.7 (0.4)	7.81 (0.29)	7.1 (0.2)	7.73 (0.11)

[a]Standard deviation.

[4]Milton Roy Pumps is a trademark of Milton Roy Co., P.O. Box 12167, St. Petersburg, FL 33733, Model 196-0015. Reference to commercial products does not constitute endorsement by the University of West Florida.

brood cups used in the mysid test. Two incubation cups were placed in each of two duplicate 2-L aquaria per treatment as described by Goodman et al [*6*]. TEG was introduced with a dosing apparatus by Schimmel et al [*11*], which delivered 1 L of seawater to each treatment at 5 cycles/h, and Hamilton Precision Liquid Dispenser 11 pumps,[5] which injected measured amounts of TEG at each cycle. Fluctuation of the water level was accomplished by self-starting siphons as previously noted. Embryo and fry survival were observed daily and mortality was recorded. To prevent injury to newly hatched fry when incubation cups were removed from a chamber, the cups were changed on the day before the expected hatch. Before removal of cups, the water level in each chamber was slowly siphoned to the tops of the petri dishes of each cup. Foods for hatched fish included (1) rotifers, *Brachionus plicatilis*, three times daily for the first five days, (2) rotifers and *Artemia* nauplii (24 to 32-h post-hydration of cysts) twice daily for the next three days, and (3) only *Artemia* nauplii twice daily until termination of the test. The test was terminated on the 28th day by killing the fish in boiling water. Each fish was weighed individually.

Nominal concentrations of TEG spanned the range from those considered acceptable in ASTM standard practices for chronic tests with both mysids [*7*] and silversides [*8*] to those which were acutely toxic. Nominal concentrations in both tests were 3.2, 10, 32, 100, 320, and 1000 mg TEG/L and a seawater control. Hereafter, we will refer only to mean measured concentrations.

TEG concentrations in test water were measured weekly (Tables 2 and 3) on a Hewlett Packard 5730 Gas Chromatograph[6] equipped with dual flame ionization detectors and a 182- by 2-mm (inside diameter [I.D]) glass column packed with Tenax®-G. C. support. Gas chromatograph operating parameters were injector temperature, 200°C; oven temperature, 200°C; detector temperature, 300°C; carrier gas helium at 25 cm^3/min; and detector gases were hydrogen at 35 cm^3/min and air at 250 cm^3/min. Quantitation of fortified seawater samples from 10 to 1000 mg/L was linear, and spiked recoveries averaged 100%. The lower detection limit was 3 mg/L for the mysid test and 10 mg/L for the tidewater silverside test. The differences in detection limits can be explained by interference peaks found in the silverside water samples that were not present in the mysid water samples. With no direct extraction of TEG from the seawater sample, all impurities from the seawater were measured by the gas chromatograph. Increased impurities can be explained by changes in seasons: from winter (mysid test) to late spring (silverside test).

For mysid data, analysis of variance and Duncan's multiple range test with

[5]Hamilton Precision Liquid Dispenser 11 is a trademark of Hamilton Company, Reno, NV. Model PLD 11. Reference to commercial products does not constitute endorsement by the University of West Florida.

[6]Hewlett Packard 5730 Gas Chromatograph of Hewlett Packard Company, Avondale, PA. Model 5730. Reference to commercial products does not constitute endorsement by the University of West Florida.

[7]Supelco Inc., Belmont, PA.

TABLE 2—*Survival and reproductive success of mysids.* Mysidopsis bahia, *(48 per treatment) continously exposed to triethylene glycol in flowing seawater (25±2°C, 20±2 parts per hundred for 23 days.*

TEG Concentration, mg/L		Survival, %	Reproductively Active Females, %	Day First Brood Released	Average Number of Young in First Brood per Female
Nominal	Measured (SD)				
Seawater control	ND[a]	77	33	18	3.2
3.2	ND[a]	71	38	19	4.2
10	10 (2)	73	34	18	4.5
32	31 (10)	63	41	19	5.7
100	102 (27)	58	48	19	5.5
320	380 (103)	67	45	19	4.7
1000	1000 (370)	58	39	20	3.8

[a]Nondetectable (<3.0 mg/L).

TABLE 3—*Survival of tidewater silversides,* Menidia peninsulae, *(64 per treatment) continously exposed to triethylene glycol in flowing seawater (25±2°C, 20±2 parts per hundred) for 28 days.*

TEG Concentration, mg/L		Survival, %			Average Wet Weight, mg	Percentage of Seawater Control Weight
Nominal	Measured	Embryos	Fry	Combined		
Seawater control	ND[a]	100	83	83	13.2	...
3.2	ND[a]	94	93	83	14.2	109
10	ND[a]	98	89	88	12.4	94
32	15 (13)	100	62	62	13.9	105
100	40 (15)	94	81	75	12.1	92
320	230 (90)	91[b]	86	78	10.8[b]	82
1000	1500 (1000)	97	55[c]	53[b,c]	9.7[b]	73

[a]Nondetectable (<10.0 mg/L).
[b]Significantly different from seawater control at ($\alpha = 0.05$).
[c]Two replicates not used in statistical analysis because of malfunction of test apparatus.

arcsine transformation were used to determine differences between controls and TEG treatments for survival, number of reproductively active females, day of first brood release, and number of young released per female in their first brood. Survival data were also analyzed by Kendall's ranking (linear regression). For silverside data, the same statistical methods were used to determine differences between controls and TEG treatments for survival of embryos and fry and for wet weight directly, and as a percentage of the control weight. Significant differences ($\alpha = 0.05$) were attributed to TEG exposure.

Results

In the entire life-cycle toxicity test with *Mysidopsis bahia*, survival, average number of young released in the first brood per female, and number of repro-

ductively active females in the TEG treatments were not significantly different from that of seawater controls (Table 2). For overall treatments, survival averaged 65% (range: 58 to 77%). Kendall's ranking coefficient, although not significant at $\alpha = 0.05$, was -0.31102. This negative number insinuates a negative trend in survival with increasing exposure concentrations.

In the early life-stage toxicity test with tidewater silversides, statistically significant differences ($\alpha = 0.05$) were found in weight and survival at the high TEG concentrations: 230 and 1500 mg/L (Table 3).

Average weight of fish exposed to 230-mg TEG/L was 18% less than average control weight, while average weight of fish exposed to 1500-mg TEG/L was 26% less than average control weight. In the 1500 mg/L treatment, the significant effect in combined survival (embryo and fry) was 36% less than that of the control. Survival of embryos was significantly different from the control at 230 mg/L but not at 1500 mg/L. This suggests that the effect observed in the 230-mg/L treatment may not be caused by toxicity of TEG because the response was not concentration dependent.

Discussion and Conclusions

In acute and chronic toxicity tests with three species of freshwater fishes by Cardwell et al [*3*], TEG was found to have a low order of toxicity (g/L). For those species, the median lethal concentrations determined under flow-through conditions were 1500 to 2300 times higher than the measured concentrations at which we observed no effect on *Menidia peninsulae*. Effects other than toxicity have been observed. TEG treatments by Taub and Harrass [*4*] (single or repeated additions to static microcosms containing freshwater species) were observed to cause gradual but persistent lowering of pH. A pH effect was not observed in our flow-through system. They showed degradation of TEG (acidic compounds) could lead to a decline in *Daphiae* populations in static systems.

Kendall's ranking for mysid survival, although a negative value, was not significant at $\alpha = 0.05$. This value was determined by using each replicate's percent survival value as individual datapoints instead of obtaining an average percent survival per concentration. This allowed for 21 datapoints versus 7 datapoints. Because the ranking value was a negative value, there is an insinuation of possible masking of effects on survival with an increase in concentration of TEG. The possible masking of survival effects may be explained by the variability between replicates of each concentration. This variability was present in all concentrations. The variability between replicates also made calculating Beta or the power of the test impossible. Therefore, we were unable to determine if a Type II error had been committed.

Even with the Kendall's ranking and the large variability, we concluded that TEG can be used as a carrier for substances in chronic toxicity tests with *Mysidopsis bahia* and *Menidia peninsulae* without direct toxicity at concentrations much higher than those suggested in ASTM drafts of standard prac-

tices ($\leq$0.01 mL/L [$\leq$0.011 mg/L] in mysid life-cycle tests and $\leq$0.1 mL/L [$\leq$0.11 mg/L] in tests with early life stages of an atherinid fish). Data presented suggest that because the toxicity of TEG appears to be 100 000 times that recommended for mysid life-cycle tests and is 360 times that recommended for early life-stage tests, either a seawater or a TEG control may not be necessary.

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